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Estimated Daily Intake of Aflatoxin M₁ in Thailand

Nongluck Ruangwises¹, Piyawat Saipan² and Suthep Ruangwises³

¹*Department of Pharmaceutical Chemistry, Faculty of Pharmacy,
Mahidol University, Bangkok*

²*Department of Veterinary Public Health, Faculty of Veterinary Medicine,
Khon Kaen University, Khon Kaen*

³*Department of Veterinary Public Health, Faculty of Veterinary Science,
Chulalongkorn University, Bangkok
Thailand*

1. Introduction

Aflatoxins are a group of mycotoxins produced by certain species of *Aspergillus*. These molds grow on a variety of food and feed commodities and produce aflatoxins under appropriate temperature and humidity (Jay et al., 2005). Aflatoxin B₁ (AFB₁) is the most potent hepatocarcinogen of this group of mycotoxins. Aflatoxin M₁ (AFM₁) is a hydroxylated metabolite of AFB₁ and is secreted in the milk of mammals that have eaten contaminated foods. AFM₁ is also a hepatocarcinogen and is classified in Group 1 (carcinogenic to humans) by the International Agency for Research on Cancer (IARC, 2002). Exposure to AFM₁ through milk products is considered to be a serious public health problem. Several countries have established regulatory limits for AFM₁ in raw milk and milk products, which vary from country to country. According to the Food and Agriculture Organization of the United Nations (FAO), there are 60 countries that have established regulatory limits for AFM₁; the values vary from ND (not detectable) to 15 µg/L (FAO, 2004). The two most prevalent limits are 0.05 µg/L (34 countries) and 0.5 µg/L (22 countries). The European Community has set the maximum permitted level for AFM₁ in infant formulae and follow-on formulae, including infant milk and follow-on milk, at 0.025 µg/kg, and in raw milk and heat-treated milk at 0.05 µg/kg (European Commission, 2006). The U.S. regulatory limit for AFM₁ is 0.5 µg/L (FAO, 2004). However, several countries, including Thailand, have not yet established regulatory limits for AFM₁. The Notification of the Ministry of Public Health No. 265 – the law that regulates the quality of milk products in Thailand – only states that “...milk products may be contaminated with aflatoxins at a level that is not harmful to human health” (Ministry of Public Health, 2003).

A national food consumption survey was conducted in Thailand during the years 2002–2004; 18,746 participants were divided into five age groups (Groups 1–5): 0–3, >3–9, >9–19, >19–65, and >65 yr (National Bureau of Agricultural Commodity and Food Standards, 2006). The survey showed that the consumption amounts of four types of milk products – milk powder, school milk, commercial pasteurized milk, and UHT (ultra-high-temperature) milk – comprised approximately 93% of all milk products consumed by the Thai population

(National Bureau of Agricultural Commodity and Food Standards, 2006). In Thailand, in the morning on every school day, students in Grades 1–6 (age range, 6–12 yr) are served pasteurized milk (200 ml) provided by the School Milk Project (Ruangwises & Ruangwises, 2009). Thailand is administratively divided into 76 provinces; the 45 provinces with the highest population were selected for milk sample collection. The purposes of this study were to investigate whether the concentrations of AFM₁ in milk powder, school milk, commercial pasteurized milk, and UHT milk products consumed in Thailand are within the acceptable level for consumption, and to estimate the daily intake of AFM₁ for the Thai population.

2. Materials and methods

2.1 Chemicals

AFM₁ reference standard (from *Aspergillus flavus*), trifluoroacetic acid, methylene chloride, *n*-hexane, and silica gel 60 were purchased from Sigma-Aldrich (St. Louis, MO, USA). AflaM₁TM immunoaffinity columns were purchased from Vicam (Nixa, MO, USA). Spherisorb ODS-2 HPLC columns (5 μ m, 4.6 \times 250 mm) and C₁₈ Sep-Pak columns were obtained from Waters Corporation (Milford, MA, USA). Solvents (HPLC grade) – acetonitrile, methanol, isopropyl alcohol, and water – were obtained from Merck (Darmstadt, Germany).

2.2 Milk sample collection and sample preparation

Milk powder samples were purchased from supermarkets (2 samples/province), while school milk samples were collected from 180 elementary schools (4 schools/province). Commercial pasteurized and UHT milk samples were purchased from supermarkets (2 samples each of pasteurized and UHT milk/province). All milk samples were collected between January 2007 and January 2008. Pasteurized milk samples were frozen at -20 °C until analysis (within one month from the manufacturing date). A total of 450 milk samples were analyzed in this study.

Milk powder sample (31.25 g) was reconstituted in 200 ml of distilled water in a 250-ml volumetric flask, mixed well, and adjusted to 250 ml with distilled water (dilution 1: 8). Concentrations of fat, protein, and solid-not-fat (SNF) in reconstituted milk powder samples, analyzed using a MilkoScan 133B (Foss Electric, Hillerød, Denmark), were 4.12 \pm 0.36, 3.21 \pm 0.08, and 8.59 \pm 0.09 g/100 ml (*n* = 30), respectively. These milk compositions conformed with the Notification of the Ministry of Public Health No. 265, which states that fluid milk with full butter fat must contain fat, protein, and SNF of at least 3.2, 2.8, and 8.25 g/100 ml, respectively (Ministry of Public Health, 2003). The densities of reconstituted and liquid milk samples were determined using 50 ml of milk sample.

2.3 Extraction and determination of Aflatoxin M₁

AFM₁ was extracted from milk samples using an AflaM₁TM immunoaffinity column. The extraction procedure was according to the manufacturer's recommendations, as previously described by Ruangwises & Ruangwises (2009). In brief, an aliquot of 50 ml of reconstituted milk powder or liquid milk sample was transferred to a 50-ml plastic centrifuge tube and defatted by centrifugation at 3,500 *g* for 20 min. After the fat was separated, the resulting skimmed milk was transferred into a 50-ml plastic syringe which was attached to an immunoaffinity column. The skimmed milk was allowed to flow into the column by gravity at a rate of 1 ml/min. The column was then washed with 20 ml of water. AFM₁ was eluted with 1.25 ml of acetonitrile: methanol (3: 2), followed by 1.25 ml of HPLC water. A total

volume of 2.5 ml of eluate was filtered through a nylon filter (0.45 µm) and used for analysis of AFM₁ using HPLC. All milk samples were analyzed in duplicate.

The complete chromatographic system (Class-LC10; Shimadzu, Kyoto, Japan) consisted of a HPLC pump (model LC-10AD), an auto injector (model SIL-10A), a column oven (model CTO-10A), and a fluorescence detector (model RF-10AXL). The HPLC conditions for analysis of AFM₁ were as follows: column – Spherisorb ODS-2; column temperature – 40°C; mobile phase – water: methanol: acetonitrile (57: 23: 20); flow rate – 1 ml/min; detector – fluorescence spectrophotometer (excitation 360 nm; emission 440 nm).

The AOAC Official Method 986.16 for detection of aflatoxins M₁ and M₂ in fluid milk (Cunniff, 1995) was performed to confirm the AFM₁ analysis using the immunoaffinity column. In brief, an aliquot of fluid milk or reconstituted milk powder sample (20 ml) was mixed with 20 ml of hot water (80°C), and AFM₁ was extracted using a C₁₈ Sep-Pak column. AFM₁ was eluted from the column with ether. The ether phase was then cleaned up using a silica gel 60 mini-column; AFM₁ was eluted with a mixture of methylene chloride: ethanol (95: 5). The eluate was evaporated to dryness under nitrogen gas; the residue was then dissolved in 0.2 ml of *n*-hexane. To convert AFM₁ to AFM_{2a}, which has a higher extinction coefficient, the solution was derivatized with 0.2 ml of trifluoroacetic acid at 40°C and then evaporated to dryness under nitrogen gas. The residue was dissolved in 2 ml of water: acetonitrile (3: 1). The final solution was filtered through a nylon filter (0.45 µm) and used for analysis of AFM₁ by HPLC. The HPLC conditions for analysis of AFM_{2a} were as follows: column – Spherisorb ODS-2 (5 µm, 4.6 × 250 mm); mobile phase – water: acetonitrile: isopropanol (80: 12: 8); flow rate – 1 ml/min; detector – fluorescence spectrophotometer (excitation 365 nm; emission 455 nm).

A combination of the two methods was used to confirm the quantification of AFM₁ extracted using the AflaM₁TM immunoaffinity column. The eluate (2.5 ml) from the immunoaffinity column was evaporated under nitrogen gas to dryness. The residue was dissolved in 0.2 ml of *n*-hexane and was quantified as described in the AOAC Official Method 986.16, with the addition of 0.2 ml of trifluoroacetic acid.

2.4 Determination of limit of quantification

The limit of quantification (LOQ) for AFM₁ was determined using the Q2B method of the U.S. Food and Drug Administration (U.S. FDA, 1996). Milk samples (50 ml) were fortified with standard AFM₁ at concentrations of 0.0125, 0.025, 0.05, 0.25, and 0.5 µg/L; blank samples were not fortified with standard AFM₁. Concentrations of AFM₁ in fortified milk samples and blank samples were quantified as described in Extraction and Determination of AFM₁ using the AflaM₁TM immunoaffinity column. Calibration curves (n = 12) were obtained by least-square linear regression analysis of the residual peak heights versus fortified AFM₁ concentrations. The calculation for LOQ was based on the standard deviation of *y*-intercepts of linear regression analysis (σ) and the slope (S) using the equation LOQ = 10 σ/S. The LOQ of the method was 0.01 µg/L and the overall recovery across the five concentrations of fortified AFM₁ was 85.6%. The precision of the method, expressed as %CV (coefficient of variation), ranged from 2.8 to 5.6%, as previously discussed by Ruangwises & Ruangwises (2009).

2.5 Statistical analysis

A randomized block experiment was used to assess the differences between AFM₁ concentrations. Duncan's multiple comparison test was applied to obtain significance levels between the four types of milk samples (*P* < 0.05). SPSS Statistics version 17.0 for Windows was used for statistical analysis.

3. Results and discussion

Concentrations of AFM₁ extracted using an immunoaffinity column with HPLC quantification were comparable to those analyzed using AOAC Official Method 986.16, and also comparable to those obtained from a combination of the two methods (extraction with an immunoaffinity column, derivatization with trifluoroacetic acid, and quantification with HPLC). Comparative results for AFM₁ concentrations in liquid milk and milk powder samples obtained from the three procedures are presented in Table 1. In this study, the average density of reconstituted milk powder and liquid milk samples was 1.03 ± 0.027 g/ml (n = 90), which was used for unit conversion. Concentrations and incidence of AFM₁ in 450 milk samples are presented in Table 2. Of the 450 samples, 288 (64.0%) were found to be contaminated with AFM₁ equal to or above the LOQ of 0.01 µg/L. The incidence of AFM₁ in milk powder, school milk, commercial pasteurized milk, and UHT milk samples was 21.1% (19/90), 71.1% (128/180), 78.9% (71/90), and 77.8% (70/90), respectively. Average concentrations of AFM₁ found in the four types of milk samples were 0.004 ± 0.009, 0.035 ± 0.028, 0.048 ± 0.034, and 0.045 ± 0.034 µg/L, respectively. In this study, statistical analysis showed that the average concentration of AFM₁ in milk powder samples was significant lower than those found in the other three milk products. The average concentration of AFM₁ in school milk samples was significantly lower than those found in the commercial pasteurized and UHT milk samples. Of the 19 positive milk powder samples, only 2 samples were contaminated with AFM₁ above the EU limit for infant milk products of 0.025 µg/kg. For school milk, commercial pasteurized milk, and UHT milk samples, 68/180 (37.8%), 25/90 (27.8%), and 29/90 (32.2%) samples, respectively, were contaminated with AFM₁ within the EU limit of 0.05 µg/kg. Concentrations of AFM₁ found in all milk samples were within the U.S. regulatory limit of 0.5 µg/kg.

Sample	AFM ₁ concentration (µg/L) *		
	AOAC Official Method 986.16	Immunoaffinity column + TFA	Immunoaffinity column
<i>Milk Powder (dilution 1:8)</i>			
Sample A	0.021	0.024	0.026
Sample A + 0.05 µg/L AFM ₁	0.027	0.028	0.033
Sample A + 0.1 µg/L AFM ₁	0.119	0.120	0.125
Sample A + 0.25 µg/L AFM ₁	0.291	0.302	0.299
Sample B	0.046	0.047	0.042
Sample C	0.031	0.040	0.038
Sample D	0.022	0.028	0.023
<i>Liquid Milk</i>			
Sample 1	0.035	0.032	0.033
Sample 1 + 0.05 µg/L AFM ₁	0.079	0.078	0.076
Sample 1 + 0.1 µg/L AFM ₁	0.127	0.122	0.129
Sample 1 + 0.25 µg/L AFM ₁	0.246	0.247	0.244
Sample 2	0.062	0.060	0.065
Sample 3	0.040	0.043	0.039
Sample 4	0.089	0.085	0.091

*All samples were analyzed in duplicate.

Table 1. AFM₁ concentrations in milk powder and liquid milk samples obtained from three procedures

Sample	Samples	Positive (%)	AFM ₁ concentration (µg/kg)		AFM ₁ incidence ¹			
			Mean ± SD	Range ²	0.010- 0.050 µg/kg	0.051- 0.075 µg/kg	0.076- 0.100 µg/kg	≥ 0.101 µg/kg
Milk powder	90	19 (21.1) ³	0.004± 0.009 ⁴ A ⁵	0.011-0.048	19 (21.1)	-	-	-
Liquid milk								
Pasteurized school milk	180	128 (71.1)	0.035±0.028 B	0.012-0.104	68 (37.8)	50 (27.8)	7 (3.9)	3 (1.7)
Pasteurized commercial	90	71 (78.9)	0.048± 0.034 C	0.014-0.109	25 (27.8)	29 (32.2)	14 (15.6)	3 (3.3)
UHT commercial	90	70 (77.8)	0.045± 0.034 C	0.012-0.112	29 (32.2)	26 (28.9)	11 (12.2)	4 (4.4)
Overall	450	288 (64.0)	0.034± 0.031	0.011-0.112	141 (31.3)	105 (23.3)	32 (7.1)	10 (2.2)

¹AFM₁ incidence of the positive samples
²Ranges of AFM₁ concentrations of the positive samples
³Numbers in parentheses are percentages of each milk product
⁴Concentrations of AFM₁ in reconstituted milk powder samples (1: 8)
⁵Different letters (A, B, C) denote significant differences between means of each milk product (P < 0.05)

Table 2. Concentrations and incidence of AFM₁ in milk powder and liquid milk samples

Since each of the five Thai population groups consumed different types of milk products, calculation of the daily AFM₁ intake for each population group was based on the mean AFM₁ concentrations in the corresponding milk products. The mean daily AFM₁ intake by each group was calculated as follows: mean AFM₁ intake (ng AFM₁/day) = (mean daily milk intake, kg/day) × (mean AFM₁ concentration in the corresponding milk products, µg/kg) × 1,000 (a unit conversion factor, ng/µg). The mean consumption amounts of milk products for the five Thai population groups were 0.429, 0.220, 0.138, 0.059, and 0.036 kg/day, respectively, while the average body weights were 10.1, 20.0, 46.2, 59.4, and 54.5 kg, respectively (National Bureau of Agricultural Commodity and Food Standards, 2006). For Group 1 (age range, 0–3 yr), a mean AFM₁ concentration in milk powder samples of 0.004 µg/kg (n = 90) was used for the calculation of daily AFM₁ intake. A mean AFM₁ concentration of 0.024 µg/kg in milk powder and school milk samples (n = 270) was used for the calculation of daily AFM₁ intake for Group 2 (>3–9 yr), while a mean AFM₁ concentration of 0.040 µg/kg in school milk, commercial pasteurized milk, and UHT milk samples (n = 360) was used for Group 3 (>9–19 yr). As school milk is consumed by students in Grades 1–6 (6–12 yr), concentrations of AFM₁ in school milk samples were used for both Groups 2 and 3. For Group 4 (>19–65 yr) and Group 5 (>65 yr), a mean AFM₁ concentration of 0.045 µg/kg in commercial pasteurized and UHT milk samples (n = 180) was used for the calculation of daily AFM₁ intake. The estimated daily intakes of AFM₁ for the five Thai population groups were 1.63, 5.29, 5.50, 2.63, and 1.62 ng/day, respectively. The estimated daily intakes of AFM₁ with respect to body weight for the five population groups were 0.16, 0.26, 0.12, 0.04, and 0.03 ng/kg BW/day, respectively.

Daily intakes of AFM₁ in various regions and countries are presented in Table 3. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) calculated that the daily AFM₁ intakes in Africa, the Middle East, Latin America, Europe, and the Far East (using the mean AFM₁ concentrations in four different milk products) were 0.002, 0.005, 0.022, 0.023, and 0.36 µg/kg, respectively (JECFA, 2001). The amounts of milk products consumed in the five regions were 0.042, 0.12, 0.16, 0.29, and 0.032 kg/day, respectively. The estimated intakes of AFM₁ in the five regions were 0.1, 0.6, 3.5, 6.8, and 12 ng/day, respectively. When AFM₁ intakes were calculated with respect to body weight (assuming 60 kg), the estimated daily intakes of AFM₁ were 0.002, 0.1, 0.058, 0.11, and 0.20 ng/kg BW/day, respectively (JECFA, 2001).

Region/Country		Milk consumption ¹ (kg/day)	AFM ₁ concentration (µg/kg)	Daily AFM ₁ intake		Reference
				ng/day	ng/kg BW/day	
	Africa	0.042	0.002	0.1	0.002	JECFA (2001)
	Middle East	0.12	0.005	0.6	0.1	JECFA (2001)
	Latin America	0.16	0.022	3.5	0.058	JECFA (2001)
	Europe	0.29	0.023	6.8	0.11	JECFA (2001)
	Far East	0.032	0.36	12	0.20	JECFA (2001)
France	age 3-14 yr	0.312	0.005-0.05 ²	NA ³	0.22	Leblanc et al. (2005)
	age >15 yr	0.229	0.005-0.05 ²	NA ³	0.09	
Brazil	children	0.360 ⁴	0.061	23.92	1.04 ⁵	Shundo et al. (2009)
	adults	0.412 ⁴	0.031	11.28	0.188 ⁵	
Spain	age 4-9 yr	0.532	0.00969 ⁶	2.63	0.21	Cano-Sancho et al. (2010)
	age 10-19 yr	0.404	0.00969	2.01	0.07	
	age 20-65 yr	0.305	0.00969	1.44	0.04	
	age >65yr	0.407	0.00969	1.94	0.05	
Thailand	age 0-3 yr	0.429	0.0047	1.63	0.16	This study
	age >3- 9yr	0.220	0.0247	5.29	0.26	
	age >9-19 yr	0.138	0.0407	5.50	0.12	
	age >19-65 yr	0.059	0.0457	2.63	0.04	
	age >65yr	0.036	0.0457	1.62	0.03	
	Overall	0.176	0.032	3.33	0.12	

¹Average milk consumption for each region or country
²AFM₁ concentrations in five milk products which were used for the calculation of AFM₁ intake for each population group (see text for details)
³NA = Data not available
⁴The values are average milk consumption amounts for both sexes calculated from milk consumption amounts by males and females in each of four population groups presented in the report
⁵Daily AFM₁ intakes were a summation of individual AFM₁ intakes from five milk products
⁶A single value of average AFM₁ concentration in three milk products was used for the calculation of daily AFM₁ intake for all four Spanish population groups
⁷Average AFM₁ concentrations in corresponding milk products for each Thai population group were used for the calculation of daily AFM₁ intakes (see text for explanation)

Table 3. Daily AFM₁ intakes in various regions/countries

Leblanc et al. (2005) used individual means of AFM₁ concentrations of five milk products – butter (0.05 µg/kg), desserts (0.05 µg/kg), cheeses (0.05 µg/kg), milk (0.005 µg/kg), and ultra-fresh dairy products (0.047 µg/kg) – and daily milk consumption to calculate daily AFM₁ intakes for two French population groups: children (3–14 yr) and adults (≥ 15 yr). Daily intakes of AFM₁ for each population group were the summation of daily AFM₁ intakes from the five milk products; the estimated AFM₁ intakes by French children and adults were 0.22 and 0.09 ng/kg BW/day, respectively. Shundo et al. (2009) used AFM₁ concentrations in different milk products to estimate daily AFM₁ intake by Brazilian children and adults in the city of Sao Paulo. Based on the mean AFM₁ concentration in powder milk samples (61 ng/kg) collected from municipal day-care centers and elementary schools, an average milk consumption of 0.412 kg/day, and a body weight of 23 kg, the estimated daily AFM₁ intake for children was 1.04 ng/kg BW. For adults, the estimated AFM₁ intake was 0.188 ng/kg BW/day, which was calculated using the mean AFM₁ concentrations in milk powder and fluid milk samples purchased from supermarkets (31 ng/kg), a daily milk consumption of 0.361 kg, and a body weight of 60 kg.

Cano-Sancho et al. (2010) used an average AFM₁ concentration of 9.69 ± 2.07 ng/kg found in three milk products (UHT milk, cheese, and yogurt samples) to estimate daily AFM₁ intake by four population groups in Catalonia, Spain. Average milk consumption for each of the four population groups – children (4–9 yr), teenagers (10–19 yr), adults (20–65 yr), and elderly (>65 yr) – was 0.532, 0.404, 0.305, and 0.407 kg/day, respectively; while the body weights were 26.2, 54.1, 73.7, and 73.3 kg, respectively. The estimated AFM₁ intakes for the four Spanish population groups were 0.21, 0.07, 0.04, and 0.05 ng/kg BW/day, respectively. This study showed that 288 (64.0%) of the 450 milk samples collected from 45 provinces of Thailand were contaminated with AFM₁ equal to or more than the LOQ of 0.01 µg/L. Daily intakes of AFM₁ in five Thai population groups, calculated using corresponding AFM₁ concentrations in milk products consumed by each population group, were comparable to those of other regions and countries. Thai children (3–9 yr) had the highest AFM₁ intake, 0.26 ng/kg BW/day, which was comparable to those in the Far East (0.20 ng/kg BW/day), France (3–14 yr; 0.22 ng/kg BW/day), Brazil (adults, 0.188 ng/kg BW/day), and Spain (4–9 yr, 0.21 ng/kg BW/day). Thailand is one of several countries that have not yet established regulatory limits for AFM₁ in raw milk and milk products. The present study and our two previous reports (Ruangwises & Ruangwises, 2009, 2010) suggest regular monitoring of raw milk and milk products, and regulatory limits for AFM₁ to ensure the quality of raw milk and milk products in Thailand.

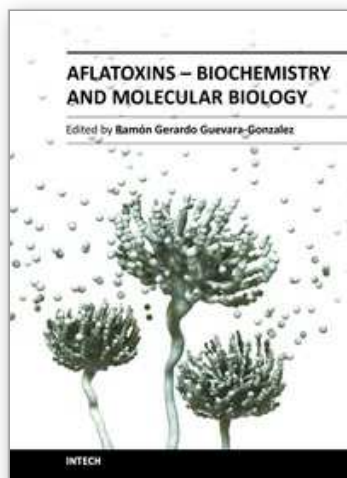
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5. References

- Cano-Sancho, G., Marin, S., Ramos, A.J., Peris-Vicente, J., & Sanchis, V. (2010). Occurrence of aflatoxin M₁ and exposure assessment in Catalonia (Spain). *Revista Iberoamericana de Micología*, Vol. 27, No. 3, (July-September 2010), pp. 130-135, ISSN 1130-1406

- Cunniff, P.A. (Ed.). (1995). *Official Methods of Analysis* (16th edition), AOAC International, ISBN 0-935584-54-4, Gaithersburg, Maryland [Section: AOAC Official Method 986.16 – Aflatoxins M₁ and M₂ in fluid milk]
- European Commission (2006). Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union*, 20.12.2006, pp. L 364/5 – L 364/24, ISSN 1725-2555
- FAO (Food and Agriculture Organization of the United Nations) (2004). *Worldwide Regulations for Mycotoxins in Food and Feed in 2003*, Food and Nutrition Paper 81, FAO, ISBN 92-5-105162-3, Rome
- IARC (International Agency for Research on Cancer) (2002). *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 82: Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene*, World Health Organization (WHO), IARC Press, ISBN 92-832-1282-7, Lyon, France
- Jay, J.M., Loessner, M.J., & Golden, D.A. (2005). *Modern Food Microbiology* (7th edition), Springer Science and Business Media, ISBN 0-387-23180-3, New York
- JECFA (Joint FAO/WHO Expert Committee on Food Additives) (2001). *Safety Evaluation of Certain Mycotoxins in Food: Aflatoxin M₁*, WHO Food Additives Series No. 47, ISBN 92-4-120940-2, Geneva, Switzerland
- Leblanc, J.C., Tard, A., Volatier, J.L., & Verger, P. (2005). Estimated dietary exposure to principal food mycotoxins from the First French Total Diet Study. *Food Additives and Contaminants, Part A*, Vol. 22, No. 7, (July 2005), pp. 652–672, ISSN 1944-0049
- Ministry of Public Health (2003). Notification of the Ministry of Public Health No. 265 (B.E. 2545): Cow's milk. *The Royal Thai Government Gazette*, Vol. 120, Special Issue 4D, (January 2003), Bangkok, Thailand (In Thai)
- National Bureau of Agricultural Commodity and Food Standards (2006). *Food Consumption Data of Thailand*, Ministry of Agriculture and Co-operatives, ISBN 974-403-423-8, Bangkok, Thailand (In Thai)
- Ruangwises, S., & Ruangwises, N. (2009). Occurrence of aflatoxin M₁ in pasteurized milk of the School Milk Project in Thailand. *Journal of Food Protection*, Vol. 72, No. 8, (August 2009), pp. 1761–1763, ISSN 0362-028X
- Ruangwises, N., & Ruangwises, S. (2010). Aflatoxin M₁ contamination in raw milk within the central region of Thailand. *Bulletin of Environmental Contamination and Toxicology*, Vol. 85, No. 2, (August 2010), pp. 195–198, ISSN 0007-4861
- Shundo, L., Navas, S.A., Lamardo, L.C.A., Ruvieri V., & Sabino, M. (2009). Estimate of aflatoxin M₁ exposure in milk and occurrence in Brazil. *Food Control*, Vol. 20, No. 7, (July 2009), pp. 655–657, ISSN 0956-7135
- U.S. FDA (United States Food and Drug Administration) (1996). *Guidance for Industry – Q2B Validation of Analytical Procedures: Methodology*, Department of Health and Human Services, Center for Drug Evaluation and Research, Rockville, Maryland, Available from: <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm073384.pdf>



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Aflatoxins – Biochemistry and Molecular Biology is a book that has been thought to present the most significant advances in these disciplines focused on the knowledge of such toxins. All authors, who supported the excellent work showed in every chapter of this book, are placed at the frontier of knowledge on this subject, thus, this book will be obligated reference to issue upon its publication. Finally, this book has been published in an attempt to present a written forum for researchers and teachers interested in the subject, having a current picture in this field of research about these interesting and intriguing toxins.

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Phone: +86-21-62489820
Fax: +86-21-62489821

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